UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dicamba Registration Standard, Three Mutagenicity Studies, Registration No. 876-36, Accession Nos. 255980, 255981, Caswell No. 295

FROM: Stephanie P. April, Ph.D. Stephanie April 6/23/85
Review Section III
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: Robert Taylor
Product Manager (25)
Registration Division (TS-767)

THRU: Clint Skinner, Ph.D. 6-26-85
Section Head III
Hazard Evaluation Division (TS-769)

and

Theodore Farber, Ph.D. 9/27/85
Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Required Action:

Review of mutagenicity studies on dicamba to fill data gaps found in the Registration Standard.

Compound: Dicamba Tox Chem: 295

Registration No. 876-36 Registrant: Velsicol

Accession No.: 255980 255981

Material Tested: Dicamba (Banvel Technical) manufactured by Velsicol and supplied by Battelle.

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Classification: All studies are acceptable.

Conclusion: In these studies dicamba was negative for mutagenicity in all the in vitro tests, except the Differential Toxicity B. subtilis/E. coli and negative in the in vitro and in vivo (UDS) studies. Metabolic activation did not produce positive results in any of these tests.

The Drosophila study was also negative for mutagenicity. There still exists the following data gap in this mutagenicity battery: a study for chromosomal aberration.

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Data Evaluation Record

Citation

Simmon, V.F. (1979) "In vitro Microbiological Mutagenicity and Unscheduled DNA Studies of Eighteen Pesticides." U.S. Environmental Protection Agency, Research Triangle Park, NC; Report No. EPA 600/1-79-041

Materials and Methods

The following microbial test systems were used: Salmonella typhimurium (TA 1535, TA 1537, TA 1538, TA 98, and TA 100) from Bruce Ames; Escherichia coli WP2; repair-deficient and proficient strains of Bacillus subtilis and (H17 and M45) and E. coli (W311 and P3478), and yeast Saccharomyces cerevisiae D_3.

The microorganisms were kept at 4 °C on minimal agar plates supplemented with biotin and histidine. The plasmid-carrying strains were supplemented with ampicillin. Each plate is grown overnight at 37 °C in nutrient broth then shaken for 3 to 4 hours.

The following substances were put in test tubes at 43 °C:

2.00 ml of 0.06% agar
0.05 ml of indicator organisms
0.50 ml of metabolic activation mixture (optional)
0.05 ml of a solution of dicamba in DMSO; negative control or positive control (known mutagens for this system).

The procedures for Ames Salmonella test were followed (McCann, et al., 1975; Ames, et al., 1973).

To test E. coli WP2, a procedure similar to the Ames Salmonella assay was used to measure the reversion of WP2 to tryptophan independence.

The yeast S. cerevisiae D_3 changes by mitotic recombination to a red-pigmented form that has a defective enzyme in the adenine metabolizing pathway. The degree of mutation is measured as directly proportional to the number of red-pigmented colonies.

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The S. cerevisiae tester strain is stored at -80 °C, then inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 30 °C with aeration. This overnight culture is centrifuged and the cells are resuspended at concentrations of ~10^8 cells/ml in 67mM phosphate buffer (pH 7.4). The following items are added to a test tube:

1.30 ml resuspended culture. 0.50 ml of metabolic
0.50 of metabolic activation mixture
0.20 ml dicamba in DSMO or DSMO alone.

This mixture is incubated at 30 °C for 4 hours on a roller drum. Serial dilutions are plated. A positive response is indicated by a dose-related increase in the absolute number of mitotic recombinants per 10^5 survivors.

DNA repair assays with E. coli W 3120/p3478 and B. subtilis H17/M45 were conducted by the methods of Rosenkrantz and Kada (1971).

When metabolic activation was required for some chemicals, Araclor 1254 was used in the Ames test and the mitotic recombination test for S. cerevisiae.

Unscheduled DNA synthesis assays were performed in an in vitro mammalian tissue culture system with and without metabolic activation (Araclor). The UDS assays were done with W1-38 cells grown in T-25 tissue culture flasks in Eagle's Basal Medium with fetal calf serum.

The positive control substance was 4-nitroquinoline-N-oxide without metabolic activation and dimethylnitrosamine with exogenous metabolic activation. The negative control was the solvent diluted in the culture medium.

Results

These in vitro bioassay tests were evaluated statistically by (1) the parametric One-Way Classification Analysis of Variance for variances that are equal for treatments, and (2) the nonparametric Kruskal-Walis One-Way Analysis of Variance for variances of the treatments that are not equal.

Dicamba tested in DNA repair (W1-38) was negative when repeated twice without metabolic activation and weak positive with metabolic activation.

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When the experiment was repeated twice with metabolic activation, dicamba was negative for mutagenic activity in the in vitro UDS response both times.

In the differential toxicity assays using repair-deficient and proficient strains of B. subtilis and E. coli chemicals should give equal zones of toxicity (inhibition) for each strain. Dicamba was positive in these tests giving a larger zone of inhibition in the repair deficient strains B. subtilis M45 and E. coli, p 3478 than in the repair proficient strains B. subtilis H17 and E. coli W3110.

Assessment of Results

Microorganisms	Without Metabolic Activation	With Metabolic Activation
1. Escherichia coli WP2	negative	negative
2. Differential Toxicity of repair-proficient and deficient micro-organisms B. subtilis/ E. coli	positive	N/A
3. Salmonella typhimurium		
TA 1535	negative	negative
TA 1537	negative	negative
TA 1538	negative	negative
TA 98	negative	negative
TA 100	negative	negative
4. UDS (W1-38)	negative	*weak positive
5. Yeast (D3)	negative	negative

* negative when repeated twice

References

J. McCann, E. Choi, E. Yamasaki, and B. N. Ames. (1975) Detection of Carcinogens as mutagens in the Salmonella microsome test; Assay of 300 chemicals. Proc. Nat. Acad. Sci. U.S.A. 72, 5135-5139

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B. N. Ames, W. E. Durston, E. Yamasaki and F. D. Lee. (1974) Carcinogens are Mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Açad. Sci U.S.A. 71, 4159-4163

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Data Evaluation Record

Citation

Mutagenesis Screening of Pesticides Drosophila, Ruby Valencia, Study No. 68-01-2474, EPA Research Triangle Park, NC; February 1981.

Materials and Methods

Technical dicamba as a suspension in 1% glucose water in feeding solution at 3 and 2000 ppm was fed to Canton-5 wild type male Drosophila melanogaster flies ("CS" stock). This stock has a low spontaneous frequency of sex-linked recessive lethals mutation average 0.15% (0.1 to 0.3%) according to this submitted report with no references.

Treated males were mated individually to untreated "FM6" females whose X-chromosome is marked yellow, white and Bar and carries a complex of stock inversions. Several matings (broods) are made to sample the entire range of male germ cell developmental stages. Sperm in various stages of development were sampled in the four broods, as follows, with no information given as to the days mated.

Brood	Stage of Development
1	Mature sperm
2	Spermatids
3	Spermatocytes
4	Spermatogonia

Concurrent negative untreated controls (unadulterated feeding solution?), were also run, as well as reference mutagens as positive controls. The reference mutagens as positive controls include: Ethyl methane sulfonate, ethylenimine, trimethyl phosphate and 1, 2-dibromoethane.

Eight thousand chromosomes from each group were examined for mutations at each dose level.

Results

Dicamba at both concentrations tested (3 and 2000 ppm in feed) was negative. Frequencies of SLRL's at 3 ppm were 8.34×10^{-4} variants and at 2000 ppm the frequency was not given but considered to be negative. Three out of the four reference mutagens tested were positive: Ethyl methane sulfonate, ethylenimine, and trimethyl phosphate.

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The ethyl methane sulfonate which was stated to be a very potent mutagen in these systems, was very weak at 2 and 4 ppm. The fourth positive control trimethyl phosphase was negative at 100 and 300 ppm but positive at 1000 ppm. DBE (1,2-dibromoethane) was negative at 5, 10, and 50 ppm. It was concluded by the investigators that the air exposure technique for DBE was possibly spotty as well as those for other compounds and the drosophilia did not get the full dose.

Conclusions

Dicamba was negative in this mutagenesis screening with Drosophila; however, this result is inconclusive because of the poor response of the positive controls.

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